

FIG. 1

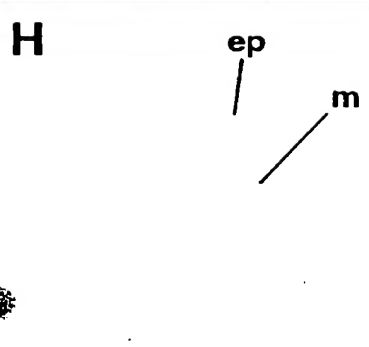
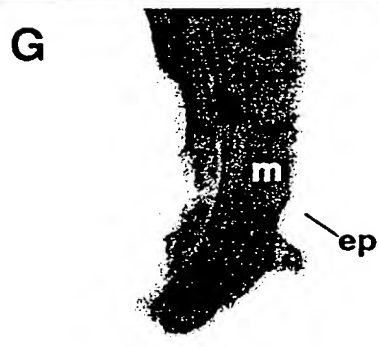
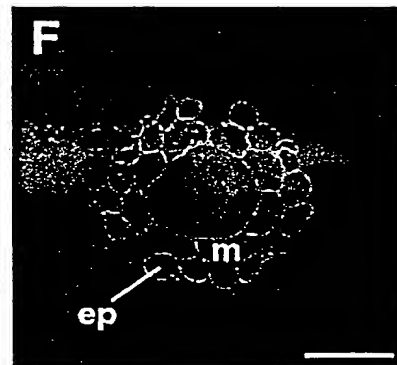
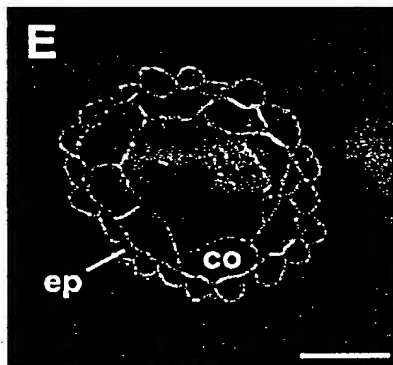
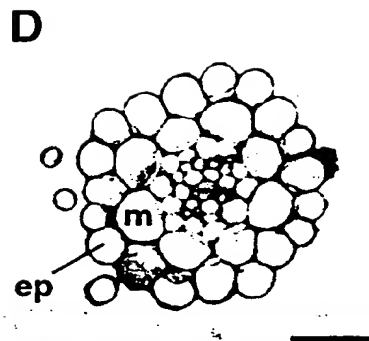
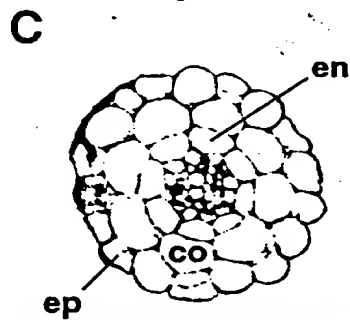
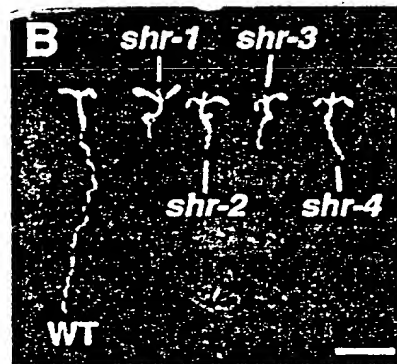
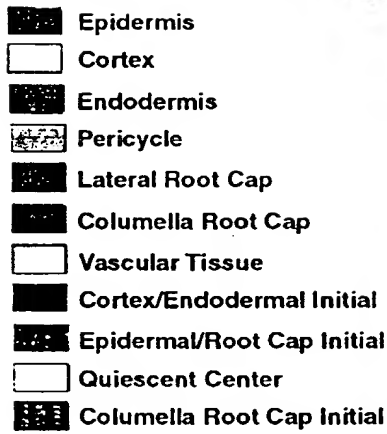
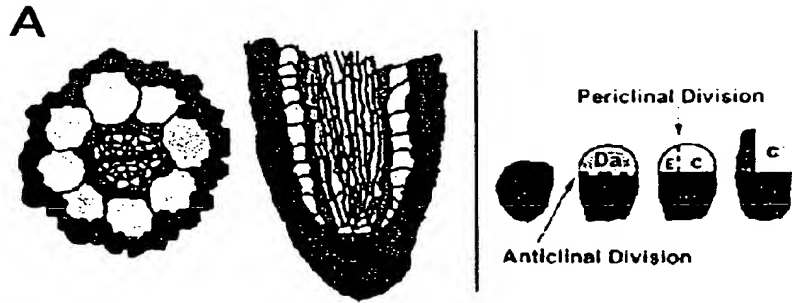


FIG. 2

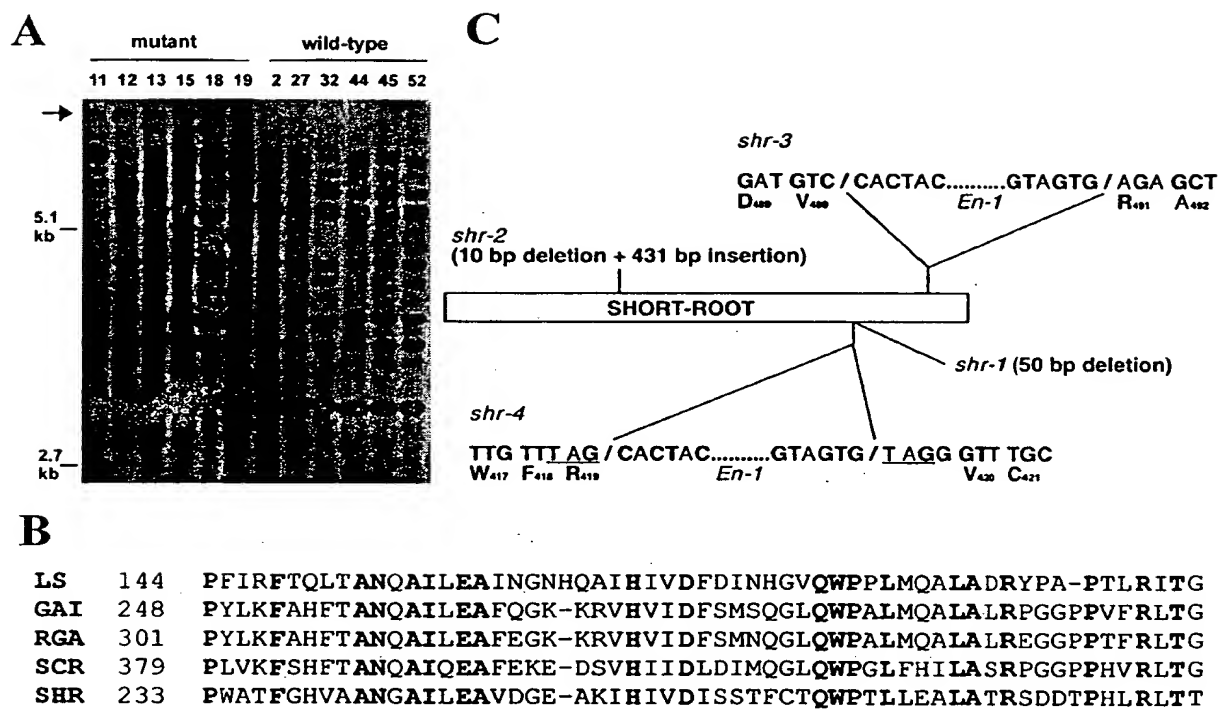
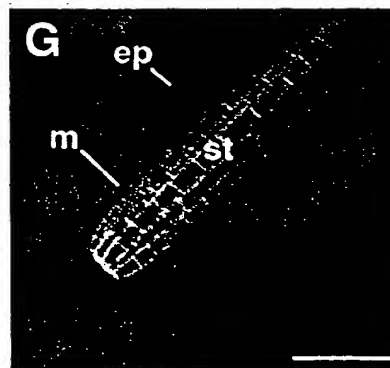
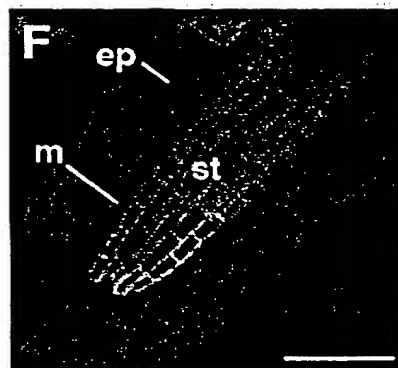
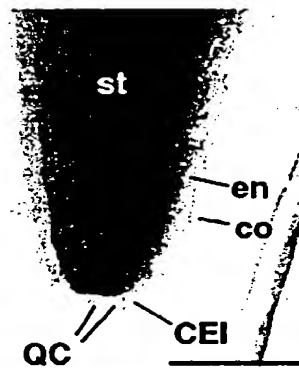
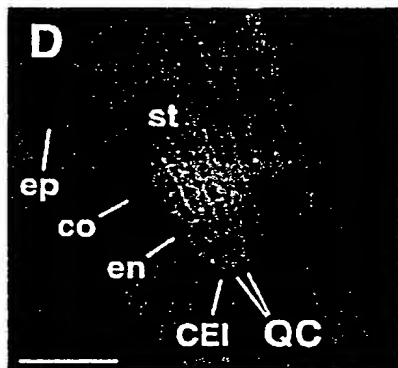
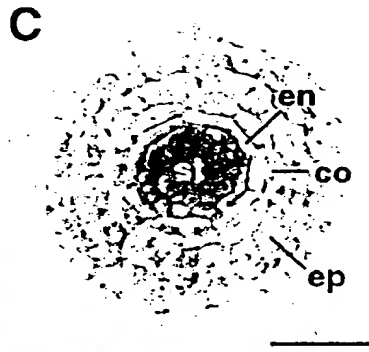
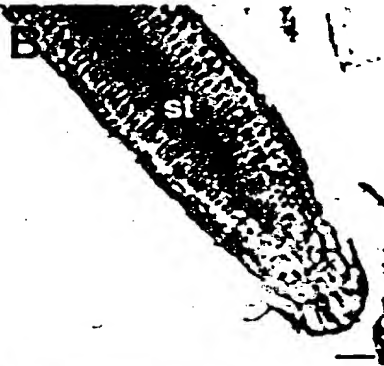


FIG. 3

A

WT *shr-1* *shr-2* *shr-3* *scr-1*

SHR



09570827.052400

FIG. 4

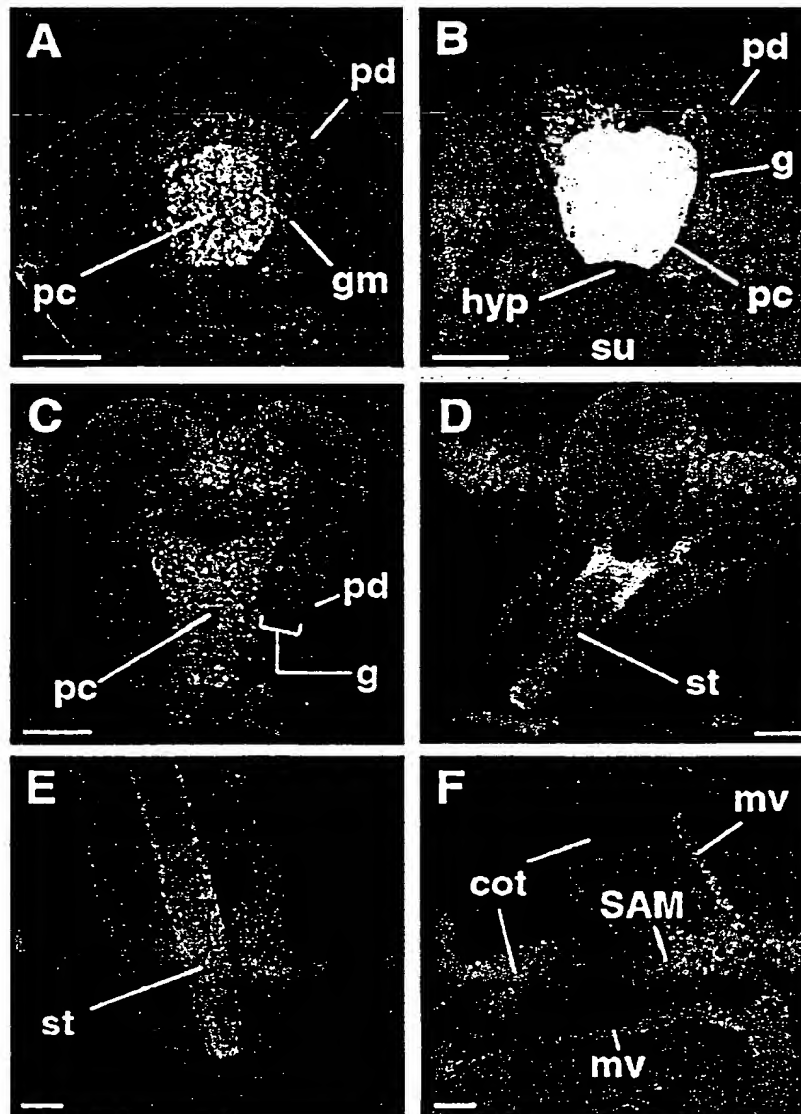


FIG. 5

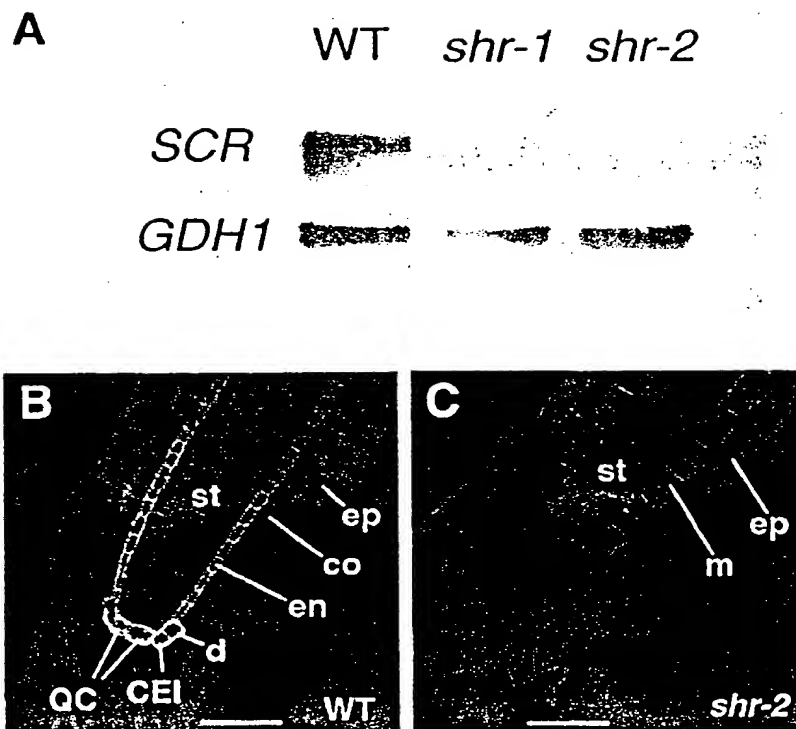
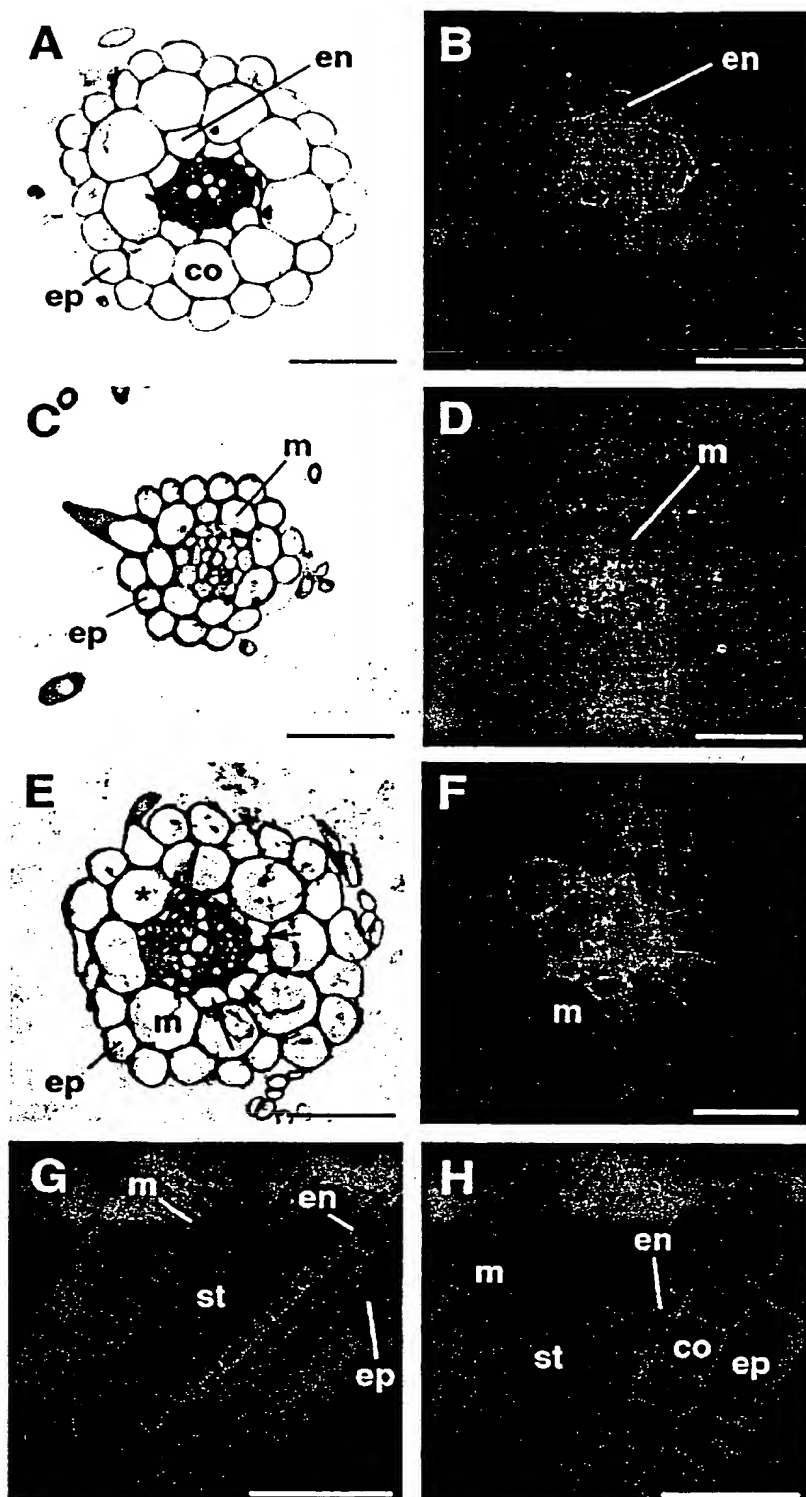
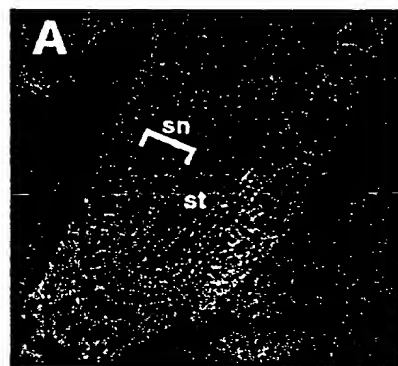


FIG. 6

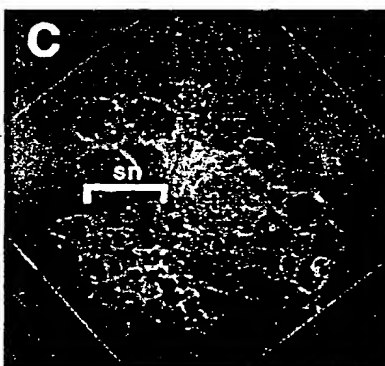


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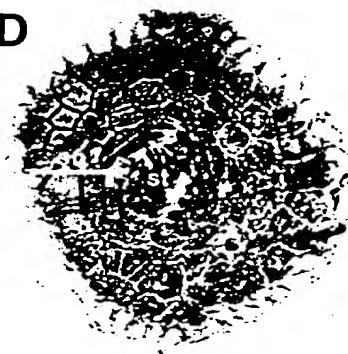
FIG. 7



**B**



**D**



**E**

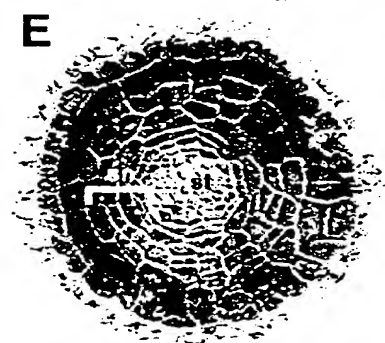


FIG. 8

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1  atcgattaag agaaaataga gttttcatgc accagtgttg atagtaacgt agtcgcggaa
61  tgtctaaaac gattatgagt ttggtggttt gattggttag aattggtatt agtaggacat
121 tctaactttt ttgtagtctt gttgatttag gatgcgtaaa gagtcttttt attttacacc
181 agttgagact tgggatcgat agtacttgaa acacttggtt ggtttcatgt atttggccta
241 tatataaaca aacatcgtaa ttatatacgg atttttttcg gaattttacg ccatactctgt
301 aagtataatat aacatgcatg tcgttttcaa attcatatga tgaacgatcc acgtaagtgc
361 tactactcct acaatattgc atgagagaga tatgtattta taaattttat ttngaagaag
421 aaataagagg gaaggttact tgggtggatc gatgtgaaaa caaagaaga aaaagcgaaa
481 cccactaagc cattacatga tatcgacctt cttatctttt tcctctttat tttatttttc
541 tcaggacttt tttctactta atgaaacctc caaactatct aactaataca ctcccatgta
601 gaataaagaa aattatataa gatattgttg atattttgta actagaaaat atatttgctc
661 tgtaattttt cgtaagttaa atcaacattt ttcagtagaa acaaatatta ctgcaaaaag
721 taggatcatt atttttgtcc aaaatctcag ttagctatag ggttgtagta aaaacaaaac
781 acattcttga tttgccccaa aaaataaaga gagagaagaa tattgttcaa aagtggctctc
841 ttctctctct aattatgttt tcaactaaac caattagatt caaacagtct acaaagtcca
901 aaagataaac atgggacaac aattcgatgc aaaaaatcct cttttcatgc tcttttttta
961 ttctctagtc ttttaatta ctaataaaaa ctacaaaatc caccaaacc cttctctaca
1021 actcaccttc atctagattt acccactccc accgagaaac acaagaaaaa aaatatacat
1081 atataaatat acaagacaac acatgatgct gatgcaatat acacaacaaa gtattaaatc
1141 ttagatattg tgggtctccc tttcttctat tcattttctt attcattaaa aaaaaaaat
1201 ggatactctc tttagactag tcagtctcca acaacaacaa caatccgata gtatcattac
1261 aaatcaatct tcgttaagca gaacttccac caccactact ggctctccac aaactgctta
1321 tcaactacaac tttccacaaa acgacgtcgt cgaagaatgc tcaactttt tctggatga
1381 agaagacctt tctcttctt cttctcacca caaccatcac aaccacaaca atcctaatac
1441 ttactactct ccttcaacta ctcccacca ataccatccc gccacatcat caacccttc
1501 ctccaccgcc gcagccgcag ctttagcctc gccttactcc tctccggcc accataatga
1561 ccttccgcg tttccatac ctcaaactcc tccgtccttc gacttctcag ccaatgccaa
1621 gtgggcagac tcggctcctt ttgaagcggc acgtgccttc tccgacaaag aactgcacg
1681 tgcgcaacaa atcctatgga cgtcacaaga gctctcttct ccgtacggag acaccgagca
1741 aaaactggct tcttaacttc tccaagctct cttcaaccgc atgaccggtt caggcgaacg
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1921 ggcaaacgga gcaatcttgg aagcagtaga cggagaggca aagatccaca tcgttgacat
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2761 ggtggttttg gctagtgcgt ggcgccaac gtaaagggtt gtttttattt tttcataagg
2821 aattc

```



## FIG. 9

MDTLFRLVSLQQQQQSDSIITNQSSLRSTTTTTGSPQTAYHYN  
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ESTRKTVLKFQEVSPWATFGHVAANGAILEAVDGEAKIHIVDISSTFCTQWPTLLEAL  
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GDLSEFDLNELDVKPDEVLAINECVGAMHGIA SRGSPRDAVISSFRRLRPRI TVVEEE  
ADLVGEEEGGFDDEFLRGFGECLRWFRVCESWEESFPRTSNERLMLERAAGRAIVDL  
VACEPSDSTERRETARKWSRRMRNSGFGAVGYSDEVADDVRALLRRYKEGVWSMVQCP  
DAAGIFLCWRDQPVVWASAWRPT

FIG. 10

```

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61  atagtatcat tacaaatcaa tcttcggttaa gcagaacttc caccaccact actggctctc
121 cacaaactgc ttatcactac aactttccac aaaacgacgt cgtcgaagaa tgcttcaact
181 ttttcatgga tgaagaagac ctttccctctt cttcttctca ccacaaccat cacaaccaca
241 acaatcctaa tacttactac tctcctttca ctactcccac ccaataccat cccgccacat
301 catcaacccc ttcctccacc gccgcagccg cagctttagc ctcgccttac tctcctccg
361 gccaccataa tgacccttcc gcgttctcca tacctcaaac tctcctcgcc tctcgactct
421 cagccaatgc caagtgggca gactcgggtcc ttcttgaagc ggcacgtgcc tctccgaca
481 aagacactgc acgtgcgcaa caaatcctat ggacgtcaa cgagctctct tctccgtaat
541 gaaaaccgct tcattttcct tgtatttgct tgaggttagg attagaccat tgggtgttac
601 tttcgaattc ttccaattta gttgttactt tcgaattctt ccatctctta gtttactaaa
661 acaaacttat gtgccccata tttctccaac aatttggtga gtggtagctt acgttttact
721 gtatacgctt ttgcagggtta tatcagcaca accattaatg atggcccggg atgtttgatg
781 ctaagatgtc ctgaccctac ttgtcttgct gctgttggtc atgatatggt tgacaaatta
841 gcgtctgaag acgaaaagga gaagtacaac agatattttc ttaggtctta tattgaagac
901 aacagaaagg taagcagtct agaaaattta tatcacacag actggtatta atgtcgtcgg
961 tcttttattg agcaaaaact ggcttcttac ttcctccaag ctctcttcaa ccgcatgacc
1021 ggttcaggcg aacgatgcta ccgaaccatg gtaacagctg cagccacaga gaagacttgc
1081 tcttcgaggt caacgcgaaa aactgtacta aagttccaag aagttagccc ctgggccacg
1141 tttggacacg tggcggcaaa cggagcaatc ttggaagcag tagacggaga ggcaaagatc
1201 cacatcgttg acataagctc cacgttttgc actcaatggc cgactcttct agaagcttta
1261 gccacaagat cagacgacac gcctcaccta aggctaacca cagttgtcgt ggccaacaag
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1441 ttatctgagt ttgatctcaa cgaactcgac gttaaaccag acgaagtctt ggccattaac
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1561 agtttccgac ggttaagacc gaggattgtg acggctcgtag aagaagaagc tgatcttgtc
1621 ggagaagaag aagggtggctt tgatgatgag ttcttgagag ggtttggaga atgtttacga
1681 tgggttaggg tttgcttcga gtcatgggaa gagagttttc caaggacgag caacgagagg
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1801 gattccacgg agaggcgaga gacagcgagg aagtggtcga ggaggatgag gaatagtggg
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1921 tataaagaag gtgtttggtc gatggtacag tgcctgatg ccgccggaat attcctttgt
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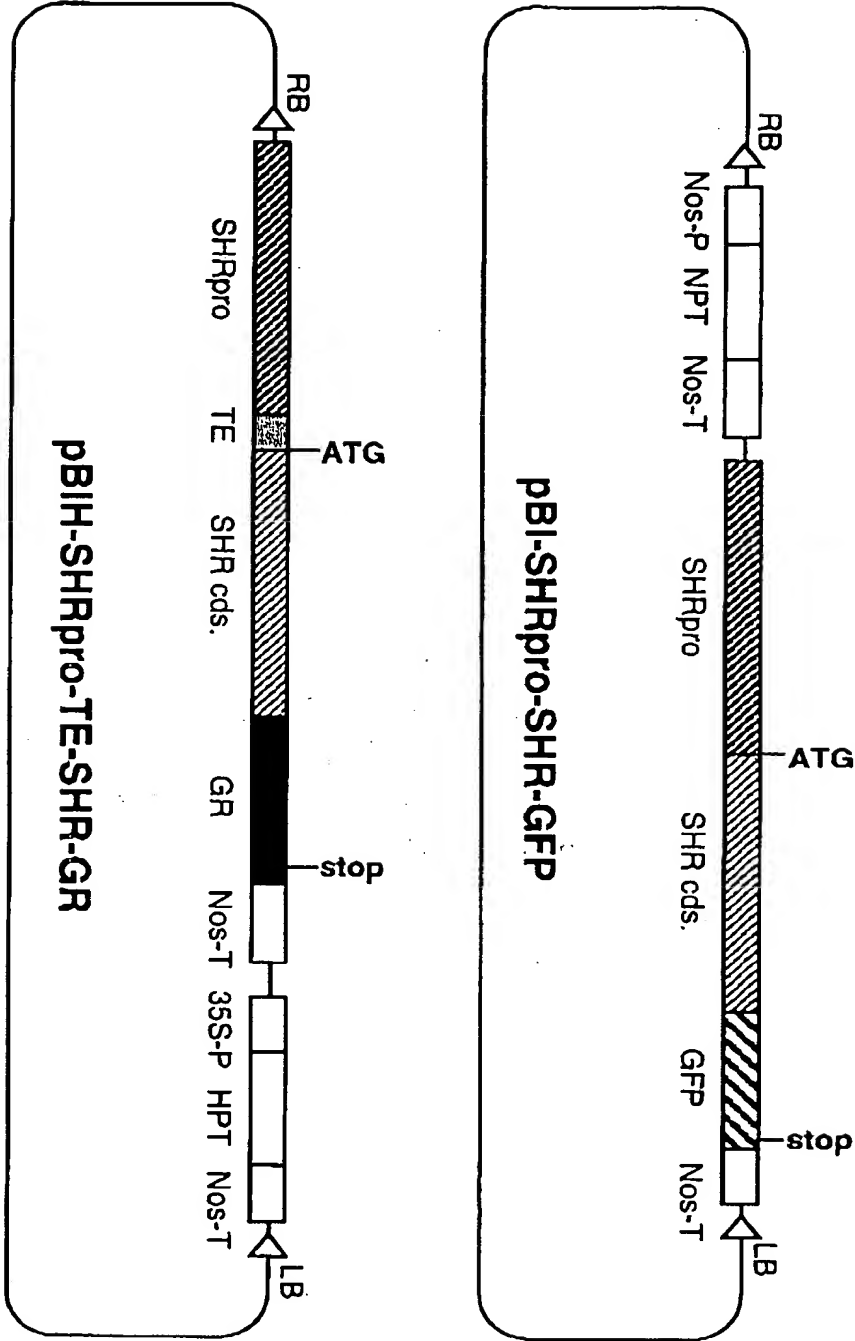
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5-kb short-root promoter sequ

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
AGAAGCAGAG	CGTGGGGTTT	CTTCTAATAA	TTGTAGAAGA	AACTGATCAT	50
GAGAACATTT	GATCTACCAG	AGATGGTGAT	GACTCATAAG	ATGTAAATAT	100
CTACTGCATT	ATGTCTAGCC	TAGGCTATAA	TGTAGATTTG	ATCACTTTCT	150
TCATTAATTA	GTTTGGAATT	TTAGCATGAT	ATAGCATATA	TCTAAATATG	200
TCCGAAACTT	TCCTACATAC	TAGAAAATAT	GGAGAGTTAT	GTAATGTAGG	250
TTTGCTTGTT	AATATACAAA	ATAACATCAT	CATTTAGTTT	TTAGATTTTT	300
TATTTTATTT	TTTATAATGG	TGCTACGTAC	GTGGCGATCA	AATTATTCCA	350
ATTTTGAGAC	TTCGGGATTT	TAAACGAAAT	TAAACAATGG	GCATGAGCTC	400
GGGGGGATAG	ACAAGATTAA	TGCTTTGTAT	CGAGACAAAC	GAGAAAATCA	450
TGATGAGCCT	ATGCATTAAG	TGCCGTTGGT	TAATTAGAGG	TTCGCATATA	500
CATAAACCAG	TAGACATATG	GATAAATATG	AACACACACA	CCAAAAAAGT	550
GGGAAATCTA	AATAAGTGTA	GAGAATAATA	AGTCCTCAGG	TGGGAGATTC	600
AAAGAGAGGA	CAATGAAGGG	TATATAGACT	CTAAACAAAA	ATGGCATGAC	650
TTAGTGGAGA	GGGTTTTTAA	TTGAAACAAG	TAGGATTGAA	GAACAAGAAA	700
ACAAAGAAGC	ATGCCCTAGA	TTTCTGAGAT	AATAATTACA	CATTGCTGTT	750
TATATAAGGT	AAGAGAATAT	GACACATTGG	TTGGTTTCTT	ACGGGTAAAT	800
GTGAAGAAAA	AAAAATAGTA	ATATTTGAGA	AAATCTAAAA	TAGTAAAGAG	850
GTATATATGG	AGAAGAAGAG	AGAAAAGGGA	AAAATAGTGG	CAGAGAATGG	900
AGAGAGGTTA	GGAGGCAAAAG	GCAAAATGTGG	AGCTTTGATG	ATGTTGATGC	950
ACGCCGTCAG	CTTTTCTTCA	CGCCTGCTCC	CACTCACTCA	CACCTATGAA	1000
CATTCTCTCT	CTATTTTATA	ATTATATTCA	CATGCTCTCTA	TGTTACTATG	1050
TAAATGGTGA	CCACTTAAGT	ATTTATATAT	CATGTATATA	TCTTATAGGT	1100
ATCATACAAA	ATGGTCATGA	AACCTTTGCA	ATTTCAATCT	ACTTGTTTCT	1150
TGTAGATGCT	AGCTTTTCAC	ATGTTTGTAA	AATTAGTCTG	GATCTGAAAT	1200
TCTTTAATTA	GCATTGTTTT	GTTGGTCAAC	GTTTAATTTT	TTGATTATTG	1250
ATGTCAAAAA	TTCAGAGCGT	TCAGAACTCT	TACACTAATT	TCTTAAAAAT	1300
AATCGATTAA	GAGAAAATAG	AGTTTTTCATG	CACCAGTGTT	GATAGTAACG	1350
TAGTCGCGGA	ATGTCTAAAA	CGATTATGAG	TTTGGTGTTT	TGATTGGTTA	1400
GAATTGGTAT	TAGTAGGACA	TTCTAACTTT	TTTGTAGTCT	TGTTGATTTA	1450
GGATGCGTAA	AGAGTCTTTT	TATTTTACAC	CAGTTGAGAC	TTGGGATCGA	1500
TAGTACTTGA	AACACTTGGT	TGGTTTTCATG	TATTTGGCCT	ATATATAAAC	1550
AAACATCGTA	ATTATATACG	GATTTTTTTT	GGAATTTTAC	GCCATATCTG	1600
TAAGTATATA	TAACATGCAT	GTCGTTTTTCA	AATTCATATG	ATGAACGATC	1650
CACGTAAGTG	CTACTACTCC	TACAATATTG	CATGAGAGAG	ATATGTATTT	1700
ATAAATTTTA	TTTTGAAGAA	GAAATAAGAG	GGAAGGTTAC	TTGGGTGGAT	1750
CGATGTGAAA	ACAAAAGAAG	AAAAAGCGAA	ACCCACTAAG	CCATTACATG	1800
ATATCGACCT	TCTTATCTTT	TTCTCTTTTA	TTTTATTTTT	CTCAGGACTT	1850
TTTTCTACTT	AATGAAACCT	CCAAACTATC	TAACATAATC	ACTCCCATGT	1900
AGAATAAAGA	AAATTATATA	AGATATTGTT	GATATTTTGT	AACTAGAAAA	1950
TATATTTGCT	CTGTAATTTT	TCGTAAGTTA	AATCAACATT	TTTCAGTAGA	2000
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GTTAGCTATA	GGGTGTAGT	AAAAACAAAA	CACATTCTTG	ATTTGCCCCA	2100
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CTCTTTTTTT	ATTCTCTAGT	CTTTTAAATT	ACTAATAAAA	ACTCACAAAT	2300
CCACCAAACC	CATTCTCTAC	AACTCACCTT	CATCTAGATT	TACCCACTCC	2350
CACCGAGAAA	CACAAGAAAA	AAAATATACA	TATATAAATA	TACAAGACAA	2400
CACATGATGC	TGATGCAATA	TACACAACAA	AGTATTAAAT	CTTAGATATT	2450
GTGGGTCTCC	CTTTCTTCTA	TTCATTTTCT	TATTCATTAA	AAAAAAAAAA	2500
TG					2502

FIG. 11

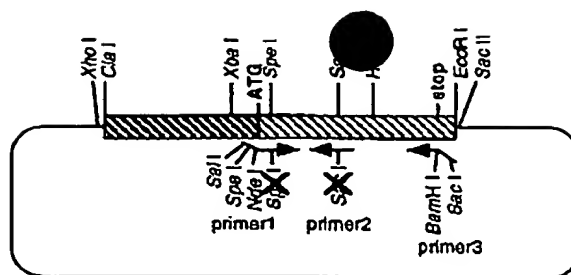
FIG. 12A



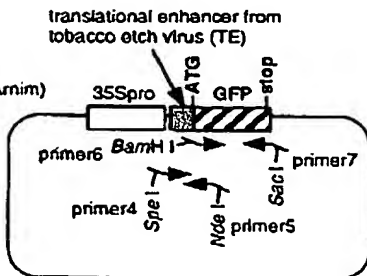
RB, right border sequence from *Agrobacterium* Ti plasmid  
SHRpro, 2.5-Kb 5' upstream region of *SHORT-ROOT* gene  
TE, translational enhancer element of tobacco etch virus  
SHR cds., *SHORT-ROOT* protein coding region  
GR, rat glucocorticoid receptor domain coding sequence  
GFP, green fluorescent protein coding sequence  
Nos-T, transcription terminator of nopaline synthetase gene  
35S-P, cauliflower mosaic virus 35S promoter  
HPT, hygromycin phosphotransferase coding sequence  
NPT, neomycin phosphotransferase coding sequence  
LB, left border sequence from *Agrobacterium* Ti plasmid

09570827 0352400

**pBS-2.8K-SHR**  
(2.8-Kb SHR genomic fragment  
in pBluescript II SK [stratagene])



**pAVA321**  
(gift from Dr. von Arnim)



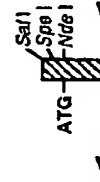
**PCR (primer4+primer5)**  
amplification of TE  
addition of *Spe* I or *Nde* I  
to each end



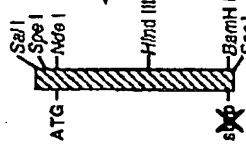
**PCR (primer6+primer7)**  
amplification of GFP  
elimination of 1st ATG  
addition of *Bam* H I or *Sac* I to each end



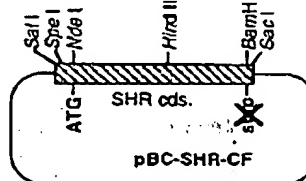
**1st. PCR (primer1+primer2)**  
elimination of internal *Sac* I  
introduction of *Nde* I at ATG  
addition of a *Sal* I-*Spe* I linker sequence



**2nd. PCR (primer1+primer3)**  
elimination of internal *Spe* I  
elimination of stop codon  
addition of a *Bam* H I-*Sac* I linker sequence

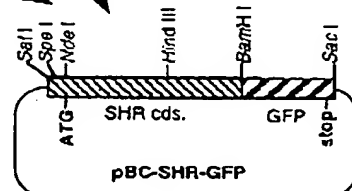
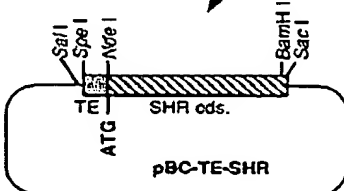


digestion with *Sal* I and *Sac* I  
ligation to pBC SK (Stratagene)



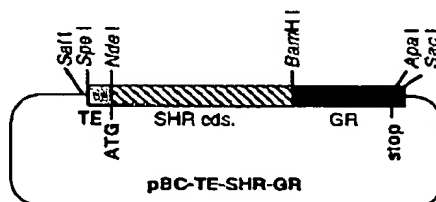
insertion with  
*Bam* H I and *Sac* I

insertion with  
*Spe* I and *Nde* I



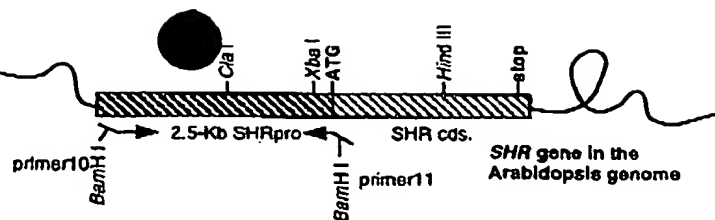
to be continued  
to PAGE 2

insertion with  
*Bam* H I and *Sac* I



to be continued  
to PAGE 3

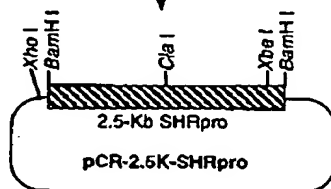
FIG. 12B



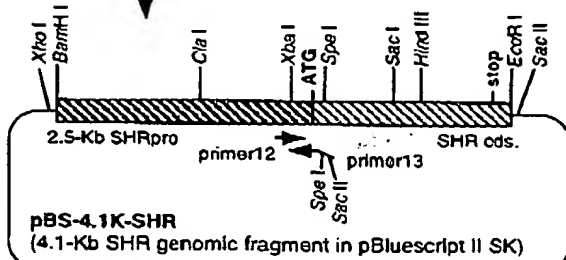
**PCR** (primer10+primer11)  
amplification of 2.5-Kb SHR promoter from  
Arabidopsis genomic DNA  
addition of *Bam*H I to both ends

TA-cloning to pCR2.1 (Invitrogen)

**pBS-2.8K-SHR**  
(shown in PAGE 1)

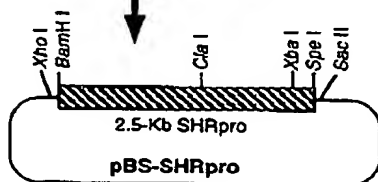


insertion with *Xho* I and *Cla* I  
(extension of the promoter region in pBS-2.8K-SHR)

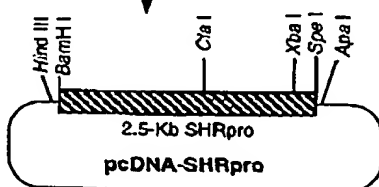


**PCR** (primer12+primer13)  
amplification of a short fragment  
from the SHR promoter  
addition of *Spe* I-*Sac* II linker sequence  
to the 3' end of the promoter

insertion with *Xba* I and *Sac* II



digestion with *Bam*H I and *Spe* I  
ligation to pcDNAII (Invitrogen)



to be continued  
to PAGE 3

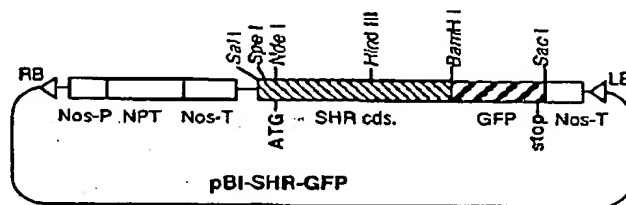
**pBI101** (clontech)

*Hind* III digestion -> Klenow -> self-ligation  
(elimination of *Hind* III site)

**pBI101ΔHind**

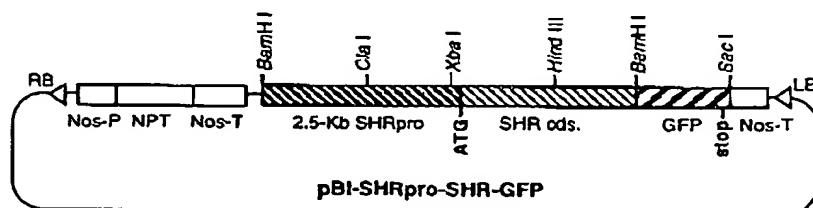
**pBC-SHR-GFP**  
(continued from PAGE 1)

insertion with *Sac* I and *Sac* I



digestion with  
*Sac* I and *Hind* III

insertion with  
*Xho* I and *Hind* III



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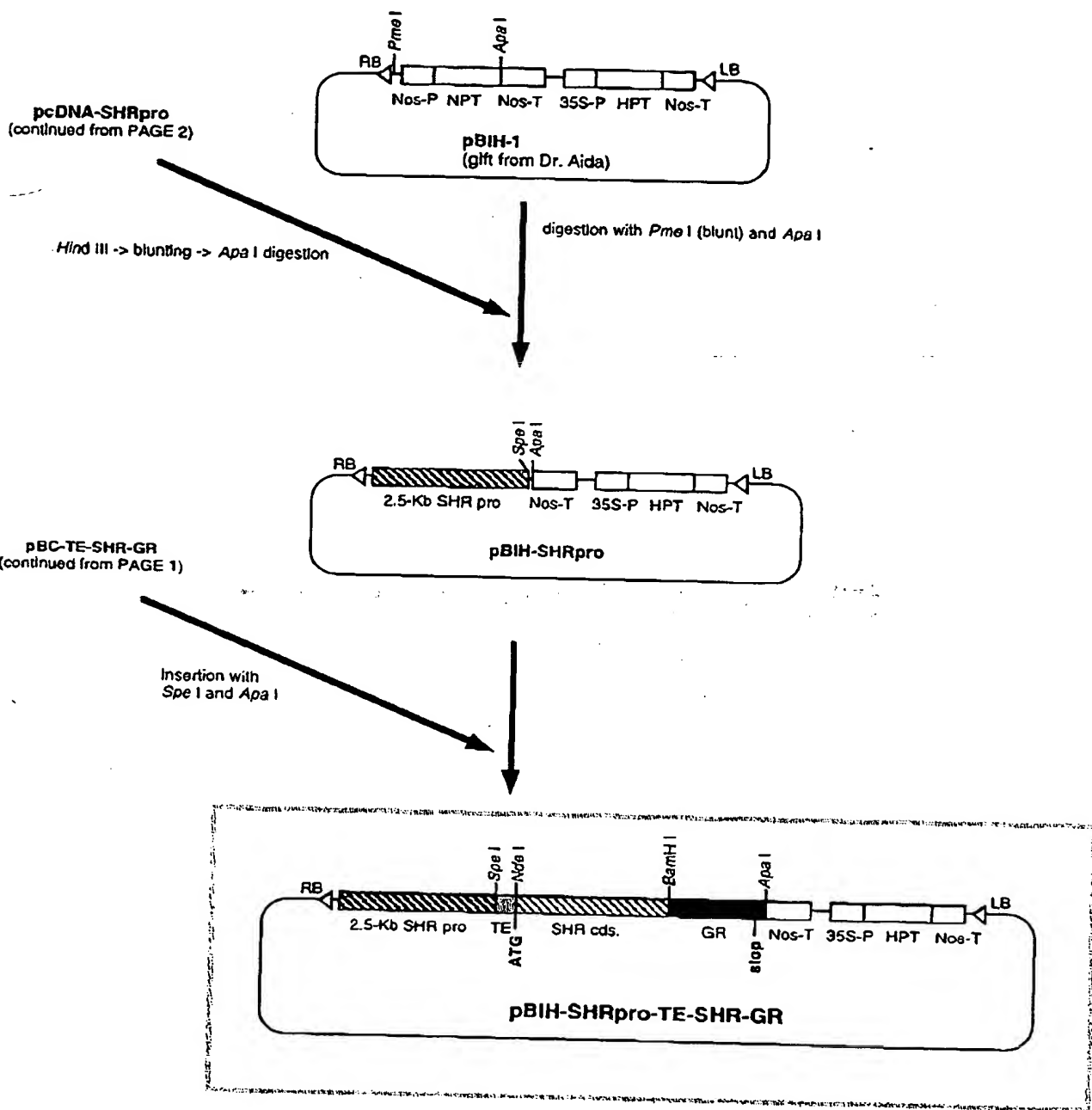
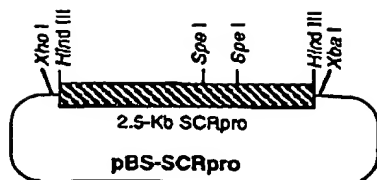
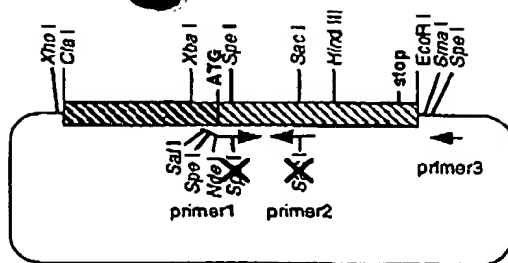
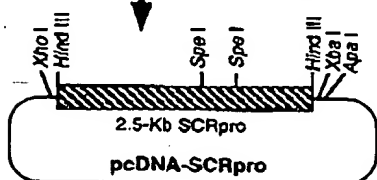


FIG. 12D

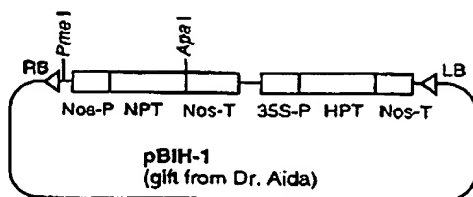
**pBS-2.8K-SHR**  
(2.8-Kb SHR genomic fragment  
in pBluescript II SK [stratagene])



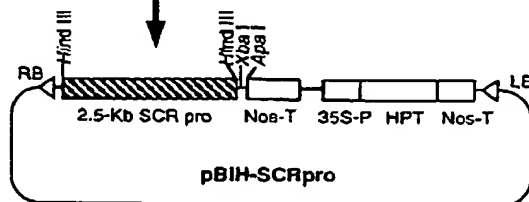
digestion with *Xho* I and *Xba* I  
ligation to pcDNAII (Invitrogen)



*Xho* I → blunting →  
*Apa* I digestion

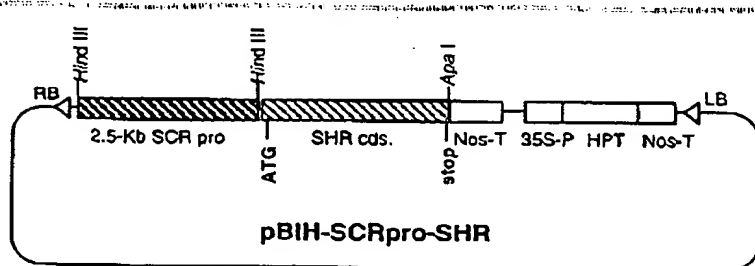


digestion with *Pme* I (blunt) and *Apa* I

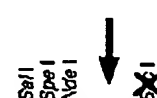


digestion with *Xba* and *Apa* I

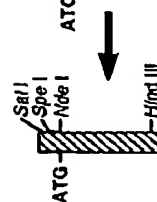
Insertion with *Spe* I and *Apa* I



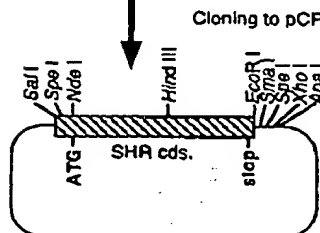
**1st. PCR** (primer1+primer2)  
elimination of internal *Sac* I and *Spe* I  
introduction of *Nde* I at ATG  
addition of a *Sal* I-*Spe* I linker sequence



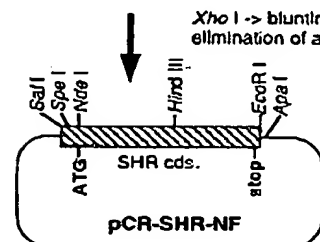
**2nd. PCR** (primer1+primer3)



Cloning to pCR2.1 vector (Invitrogen)

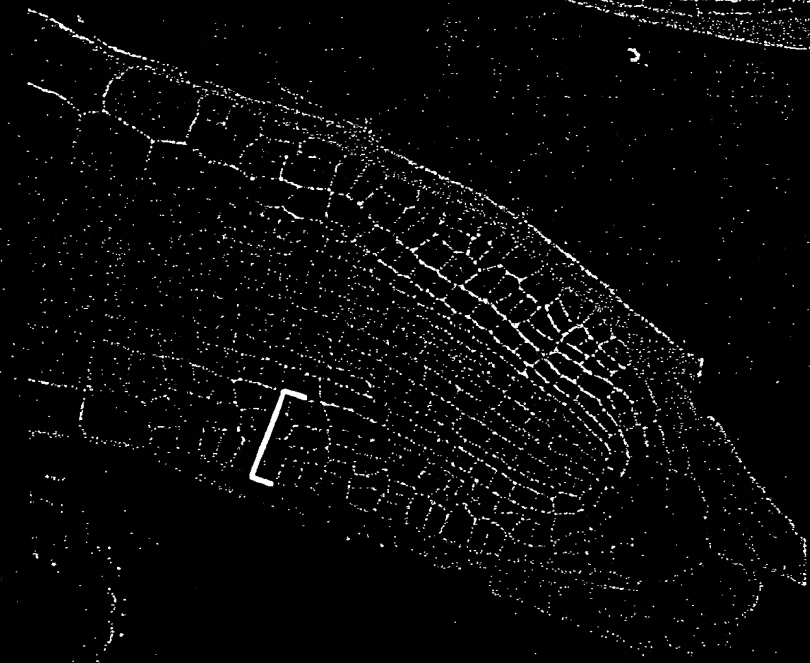


*Xho* I → blunting → *Sma* I digestion  
elimination of a downstream *Spe* I site



**FIG. 13**

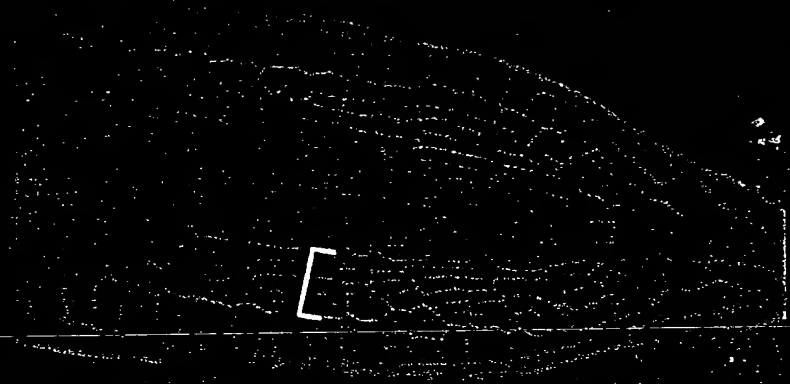




35Spro::SHR



WT



SCRpro::SHR

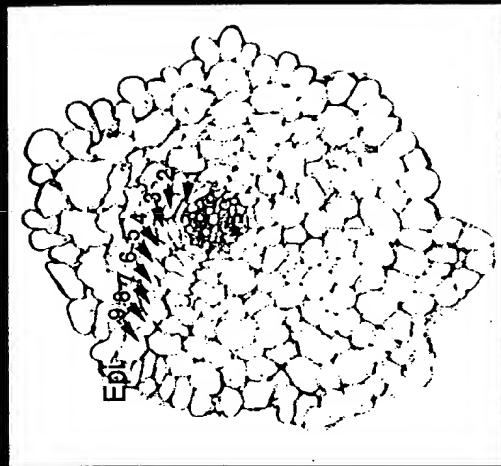
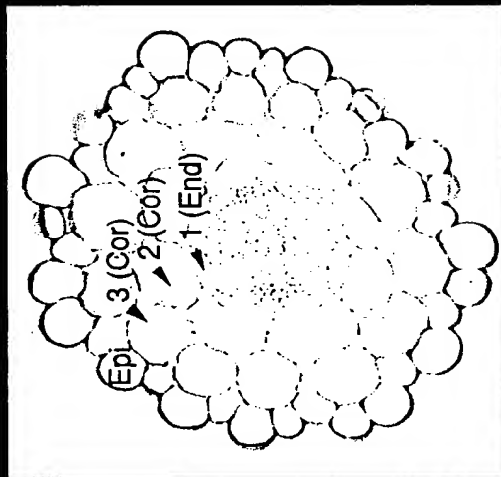
Ectopic *SHR* expression caused abnormal root cell divisions

FIG. 14

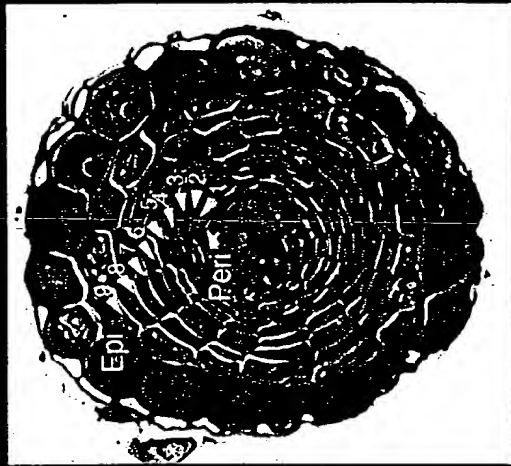
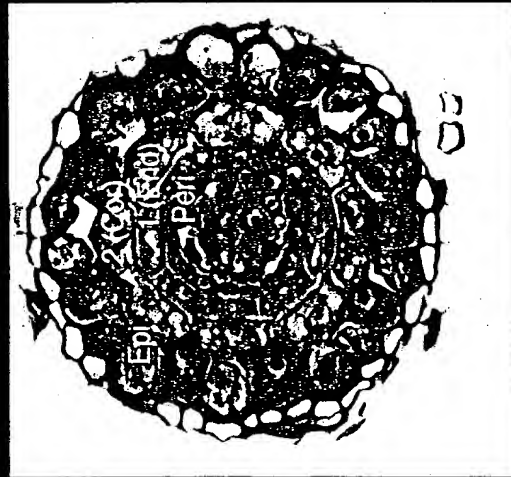
WT

*SCRpro::SHR* transgenic

hypocotyl

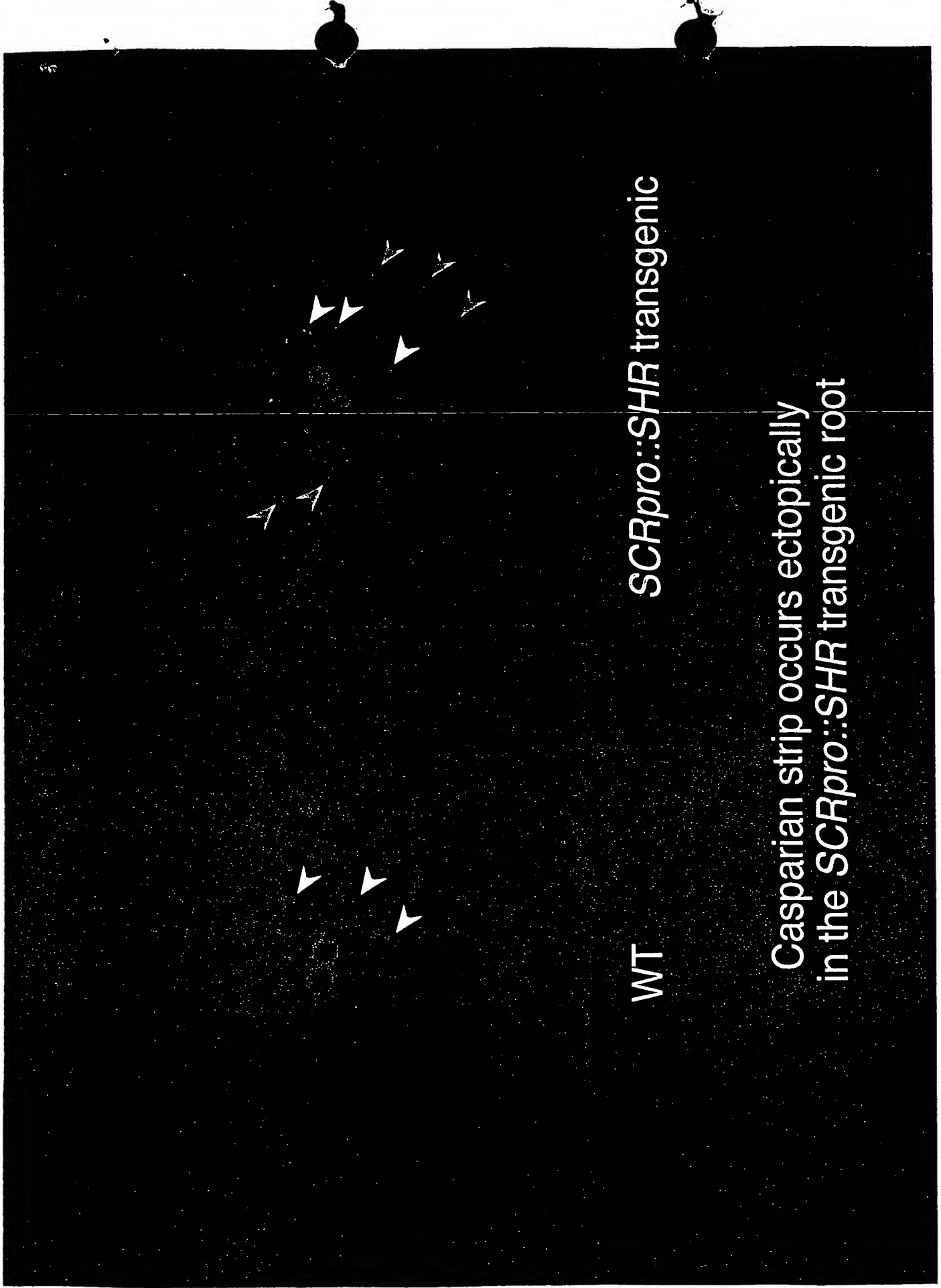


root



Ectopic *SHR* expression under the *SCR* promoter resulted in the indeterminate cell divisions in ground tissue.

FIG. 15



WT

*SCRpro::SHR* transgenic

Casparian strip occurs ectopically  
in the *SCRpro::SHR* transgenic root

FIG. 16